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Analysis of bioactive chemical compounds of *Euphorbia lathyrus* using gas chromatography-mass spectrometry and Fourier-transform infrared spectroscopy

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The aim of this study was determination the phytochemical composition of methanolic seeds extract of *Euphorbia lathyrus*. Gas chromatography-mass spectrometry (GC-MS) analysis of *E. lathyrus* revealed the existence of the Carbonic acid, (ethyl)(1,2,4-triazol-1-ylmethyl) diester, 1H-Pyrrole,2,5-dihydro-1-nitroso, Hexanal dimethyl acetal, Isosorbide dinitrate, DL-Arabinose, Cyclopropane,1-fluoro-1-(2-bromoethenyl)-2,2,3,3-tetramethyl, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-ß-d-fruc, Desulphosinigrin, D-Glucose, 6-O-α-D-galactopyranosyl, Octanoic acid, Benzofuran,2,3-dihydro, 6-Acetyl-ß-d-mannose, Estragole, Ascaridole epoxide, 3-Allyl-6-methoxyphenol, 4-Amino-1,5,pentandioic acid, l-Gala-l-ido-octonic lactone, y-Sitosterol, Tetradecanoic acid, l-(+)-Ascorbic acid 2,6-dihexadecanoate, Estra -1,3,5(10)-trien-17ß-ol, Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl), Cis-13-Eicosenoic acid, Eicosanoic acid, 3-Pyrinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10, Oleic acid, eicosyl ester, Butanoic acid, 4-chloro-,1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a, Ethyl iso–allocholate, Ethyl iso –allocholate, Olean-12-ene-3,15,16,21,22,28-hexol, (3ß,15α,16α,21ß,22α)- and 2,4,6-Decatrienoic acid,1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihy. The Fourier-transform infrared spectroscopy (FTIR) analysis of *E. lathyrus* seeds proved the presence of alkenes, aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters, nitro compounds, alkanes, hydrogen bonded alcohols, and phenols.

Key words: Gas chromatography-mass spectrometry (GC/MS), bioactive compounds, Fourier-transform infrared spectroscopy (FT-IR), *Euphorbia lathyrus*.

INTRODUCTION

Medicinal plant parts (roots, leaves, branches/stems, barks, flowers, and fruits) are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins (Cai et

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al., 2004; Altameme et al., 2015a; Al-Marzoqi et al., 2015). The seed of *Euphorbia lathyris* is a traditional Chinese medicine which has been used for the treatment of hydropsy, ascites, anuresis and constipation, amenorrhea, and scabies (Liu et al., 2011). Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Park and Pezzutto, 2002; Corro et al., 2014; Hameed et al., 2015a). In recent years, it was reported that the seeds of *Euphorbia* had a significant effect on leukemia, esophageal carcinoma, and skin cancer (Tapiero et al., 2002; Liu et al., 2011; Al-Marzoqi et al., 2016). The seed of *E. lathyris* is a kind of toxic traditional Chinese medicine, which is characterized by pungent, warm and poisonous in drug properties. It shows several side effects, such as irritation and inflammation intense on the skin, mouth and gastrointestinal tract irritation, carcinogenic, etc. (Buenz et al., 2004; Altameme et al., 2015b). The objective of this study was to analyse the chemical composition of seeds extract from methanol. The phytochemical compound was screened by gas chromatography-mass spectrometry (GC-MS) and Fourier-transform infrared spectroscopy (FT-IR) technique.

MATERIALS AND METHODS

Plant and preparation of extracts

E. lathyrus dried seeds were purchased from local market in hilla city, middle of Iraq. After thorough cleaning and removal of foreign materials, the fruits were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use. About 30 g of the plant sample powdered were soaked in 100 ml methanol for 16 h in a rotatory shaker (Hamza et al., 2015; Hussein et al., 2016a). Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture (Altameme et al., 2015c; Hameed et al., 2015b).

Identification of component by GC-MS analysis

The physicochemical properties of *E. lathyrus* are shown in Table 1. Interpretation of mass spectroscopy (GC-MS) was conducted by using data base of National Institute Standard and Technology (NIST) having more than 62000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST library. The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures (Hadi et al., 2016; Hameed et al., 2015c; Hussein et al., 2016b).The GC-MS analysis of the plant extract was made in an Agilent 7890 A instrument under computer control at 70 eV. About 1 µl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. The fragments obtained were actually charged ions with a certain mass (Hameed et al., 2015d; Hussein et al., 2016c). Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1 ml/min. The electron gun of mass detector liberated electrons having energy of about 70 eV. The column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane).

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of *E. lathyrus* specimen was treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). The sample was run at infrared region between 400 and 4000 nm (Hussein et al., 2016; Jasim et al., 2015).

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic seed extract of *E. lathyrus* shown in Table 1. The GC-MS chromatogram of the 31 peaks of the compounds detected is as shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *E. lathyrus* showed the presence of thirty one major peaks and the components corresponding to the peaks were determined as follows. The first set up peak was determined to be Carbonic acid, (ethyl)(1,2,4-triazol-1-ylmethyl) diester (Figure 2). The next peaks were considered to be 1H-Pyrrole,2,5-dihydro-1-nitroso, Hexanal dimethyl acetal, Isosorbide dinitrate, DL-Arabinose, Cyclopropane,1-fluoro-1-(2-bromoethenyl)-2,2,3,3-tetramethyl, α-D-Glucopyranoside, O-α-D-glucopyranosyl - (1.fwdarw.3) – ß - d-fruc, Desulphosinigrin, D-Glucose, 6-O-α-D-galactopyranosyl, Octanoic acid, Benzofuran,2,3-dihydro, 6-Acetyl-ß-d-mannose, Estragole, Ascaridole epoxide, 3-Allyl-6-methoxyphenol, 4-Amino-1,5,pentandioic acid, l-Gala-l-ido-octonic lactone, y-Sitosterol, Tetradecanoic acid, l-(+)-Ascorbic acid 2,6-dihexadecanoate, Estra-1,3,5(10)-trien-17ß-ol, Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl), Cis-13-Eicosenoic acid, Eicosanoic acid, 3-Pyrinecarboxylic acid , 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10, Oleic acid, eicosyl ester, Butanoic acid, 4-chloro-,1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a, Ethyl iso-allocholate, Ethyl iso – allocholate, Olean-12-ene-3,15,16,21,22,28-hexol, (3ß,15α,16α,21ß,22α)- and 2,4,6-Decatrienoic acid,1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihy (Figures 3 to 31). The FTIR analysis of *E. lathyrus* seeds proved the presence of alkenes, aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters, nitro compounds, alkanes, hydrogen bonded alcohols and phenols which shows major peaks at 837.11, 918.12, 1037.70, 1145.72, 1232.51, 1261.45, 1317.38, 1409.96, 1519.91, 1625.99, 1741.72, 2682.98, 2854.65, 2924.09, 3082.25, and 3275.13 (Table 2 and Figure 32). *E. lathyris* L. active for disinfection is an herbaceous plant of Euphorbiaceae and has been extensively researched in the field of medicine. Phenolic compounds were isolated and identified from *E. lathyrus* using RP-HPLC under the

Table 1. Major phytochemical compounds identified in methanolic extract of *Euphorbia lathyrus*.

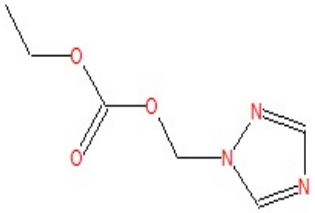
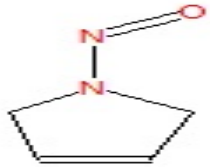
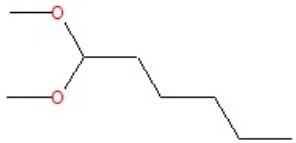
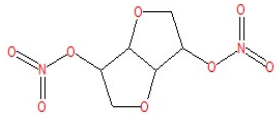
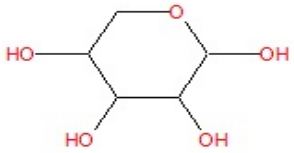
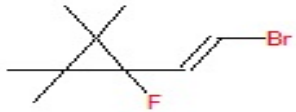
S/N	Phytochemical compound	RT (min)	Molecular Weight	Exact mass	Chemical structure	Pharmacological actions
1	Carbonic acid, (ethyl)(1,2,4-triazol-1-ylmethyl) diester	3.259	171	171.064391		Pharmacological activities such as anti-microbial, anti-inflammatory
2	1H-Pyrrole,2,5-dihydro-1-nitroso-	3.367	98	98.0480127		Antibacterial, antiviral, anticonvulsant and analgesic
3	Hexanal dimethyl acetal	3.533	146	146.13068		Antiviral and anti-inflammatory
4	Isosorbide dinitrate	4.546	236	236.028066		Relaxation
5	DL-Arabinose	4.878	150	150.052823		Anti-tumor activity
6	Cyclopropane,1-fluoro-1-(2-bromoethenyl)-2,2,3,3-tetramethyl-	5.010	220	220.026291		Many medicinal activities, which included anti-cancer and anti-cardiovascular

Table 1. cont'd

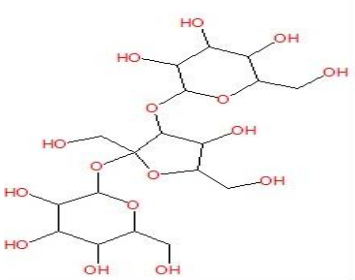
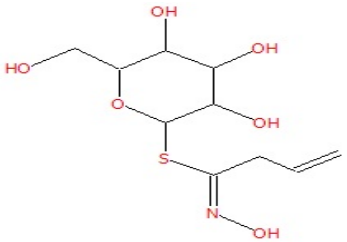
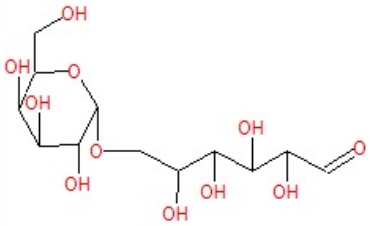
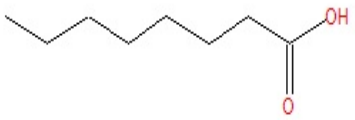
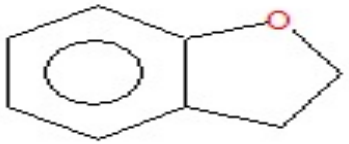
7	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-ß-d-fruc	5.261	504	504.169035		Anti-diabetic activity and anti-tumour
8	Desulphosinigrin	5.313	279	279.077658		Antibacterial activity
9	D-Glucose, 6-O-α-D-galactopyranosyl	5.782	342	342.11621		Anti-trypanosomal activity
10	Octanoic acid	6.137	144	144.115029		Anti-bacterial activity
11	Benzofuran,2,3-dihydro-	6.715	120	120.0575147		Anti-HIV, anticancer, antibacterial, and antifungal activities

Table 1. cont'd

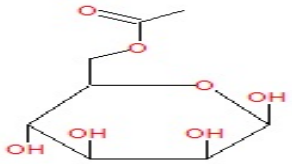
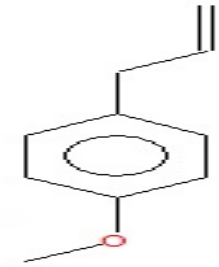
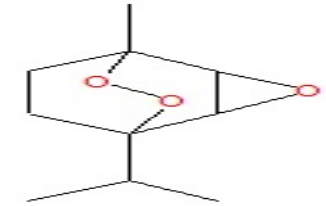
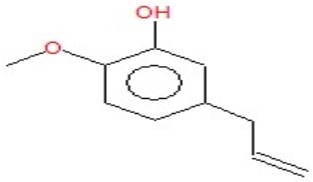
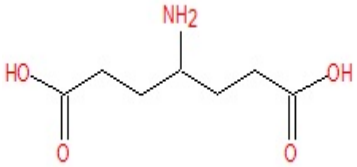
12	6-Acetyl-ß-d-mannose	6.984	222	222.073953		Anti-inflammatory and anti-oxidant
13	Estragole	7.481	148	148.088815		Estragole has been shown to have an anti-inflammatory activity
14	Ascaridole epoxide	7.727	184	184.109944		Anti-inflammatory
15	3-Allyl-6-methoxyphenol	8.443	164	164.08373		Anticancer activity
16	4-Amino-1,5,pentandioic acid	9.564	175	175.084458		Anti-cancer agent

Table 1. cont'd

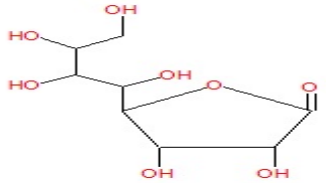
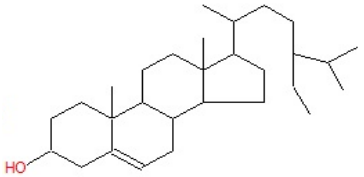
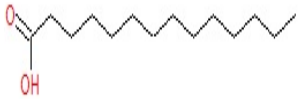
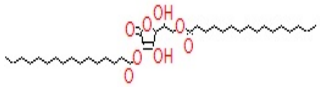
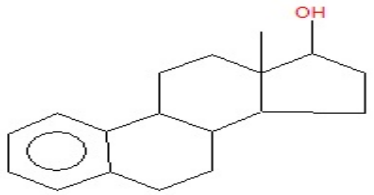
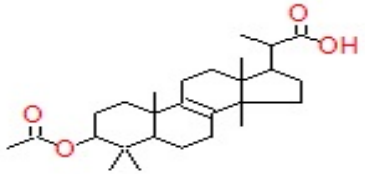
17	l-Gala-l-ido-octonic lactone	10.734	238	238.068868		Antibacterial activity against Pseudomonas aerogenosa
18	y-Sitosterol	15.023	414	414.386166		Anti-inflammatory activity
19	Tetradecanoic acid	13.455	228	228.20893		Antimicrobial, antispasmodic and *anti*-inflammatory effects
20	l-(+)-Ascorbic acid 2,6-dihexadecanoate	15.486	652	652.49142		antioxidant
21	Estra -1,3,5(10)-trien-17ß-ol	16.007	256	256.182714		New chemical compound
22	Propanoic acid ,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	16.745	430	430.30831		Anti-microbial and anti-tumor effects.

Table 1. cont'd

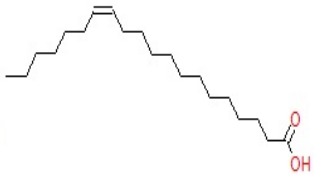
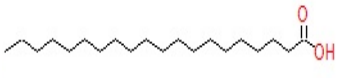
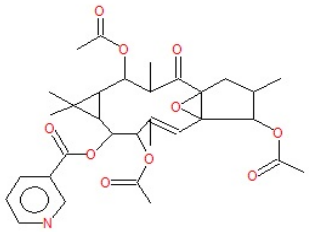
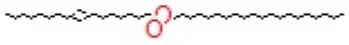
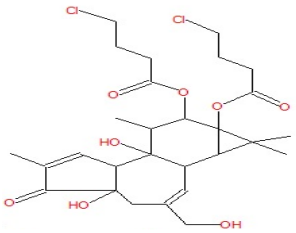
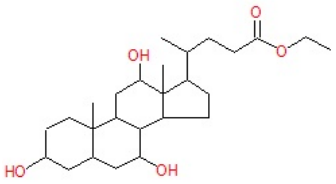
23	Cis-13-Eicosenoic acid	18.914	310	310.28718		Anti-inflammatory activity
24	Eicosanoic acid	19.051	312	312.30283		Anti-inflammatory effects
25	3-Pyrinecarboxylic acid , 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10	19.246	597	597.257397		New chemical compound
26	Oleic acid, eicosyl ester	20.339	562	562.568882		Anti-Candida activity
27	Butanoic acid, 4-chloro-,1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a	21.083	572	572.194374		Antiviral, antitumor, anti-HIV and antinociceptive activities
28	Ethyl iso –allocholate	21.134	436	436.318874		Anti-inflammatory activity

Table 1. cont'd

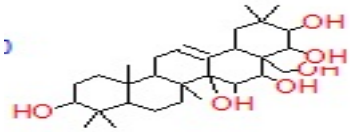
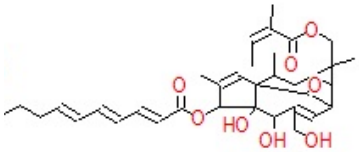
29	Olean-12-ene-3,15,16,21,22,28-hexol, (3ß,15α,16α,21ß,22α)-	21.603	506	506.360739		Anti-inflammatory
30	2,4,6-Decatrienoic acid ,1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihy	21.878	594	594.319267		Anticancer

Table 2. FT-IR peak values of *Euphorbia lathyrus*.

No.	Peak (Wave number cm^{-1})	Intensity	Bond	Functional group assignment	Group frequency
1	659.66	59.626	-	Unknown	-
2	837.11	74.522	C-H	Alkenes	675-995
3	898.83	73.438	C-H	Alkenes	675-995
4	918.12	73.336	C-H	Alkenes	675-995
5	1037.7	54.275	C-F stretch	Aliphatic fluoro compounds	1000-1050
6	1145.7	65.485	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
7	1232.5	69.798	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
8	1261.5	72.924	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
9	1317.4	73.54	NO2	Nitro Compounds	1300-1370
10	1377.2	73.54	C-H	Alkanes	1340-1470
11	1410	74.741	C-H	Alkanes	1340-1470
12	1456.3	74.929	C-H	Alkanes	1340-1470
13	1519.9	70.721	-	Unknown	-
14	1626	62.255	-	Unknown	-
15	1741.7	79.565	-	Unknown	-
16	2683	92.491	-	Unknown	-
17	2854.7	80.11	C-H	Alkanes	2850-2970
18	2924.1	73.299	C-H	Alkanes	2850-2970
19	3082.3	86.714	H-O	H-bonded H-X group	2500-3500
20	3275.1	79.255	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600

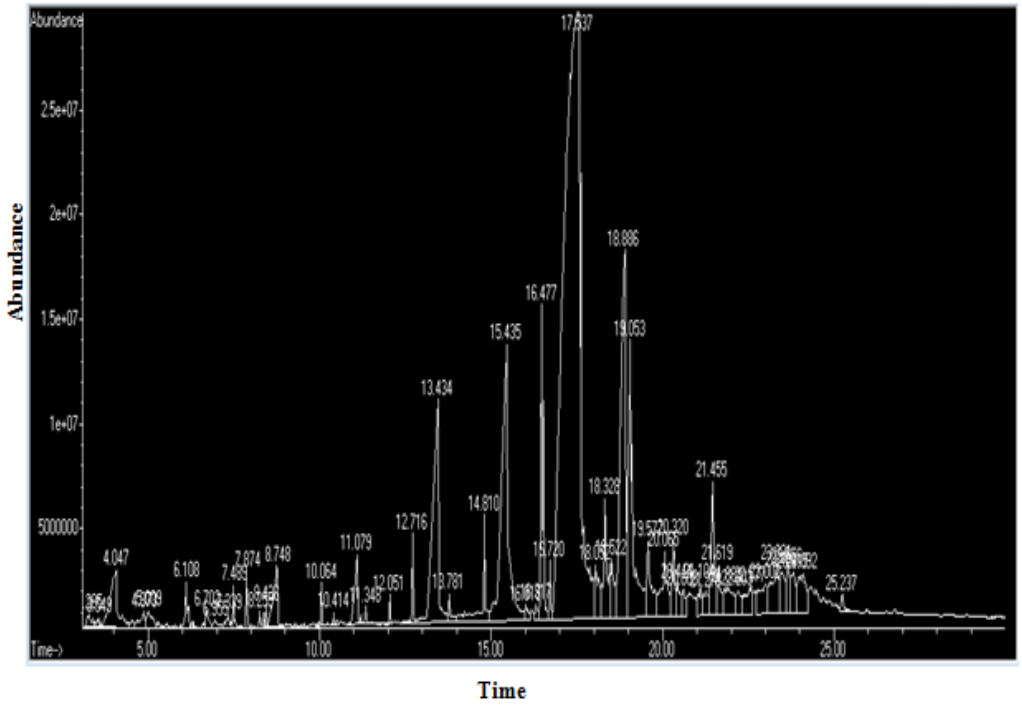


Figure 1. GC-MS chromatogram of methanolic extract of *Euphorbia lathyrus*.

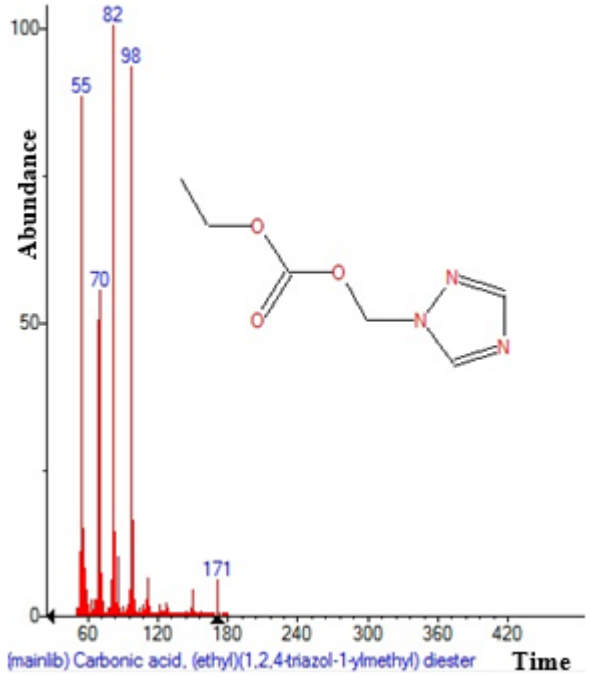


Figure 2. Structure of Carbonic acid , (ethyl)(1,2,4-triazol-1-ylmethyl) diester with 3.259 (RT) present in *Euphorbia lathyrus*.

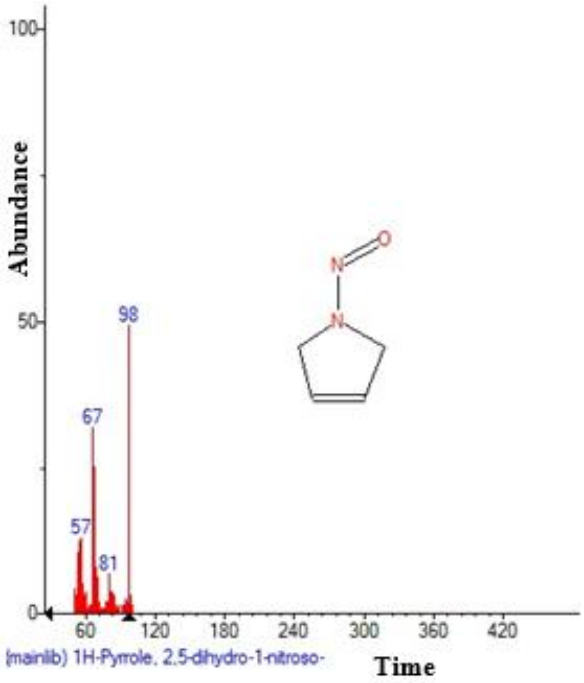


Figure 3. Structure of 1H-Pyrrole ,2,5-dihydro-1-nitroso with 3.367 (RT) present in *Euphorbia lathyrus*.

chromatographic conditions (Shahat et al., 2003; Reddy et al., 2003). *E. lathyris* L. oil (ELO) contains large amounts of FFAs and needs to determine acid value (Wei et al., 2007; Liu et al., 2011).

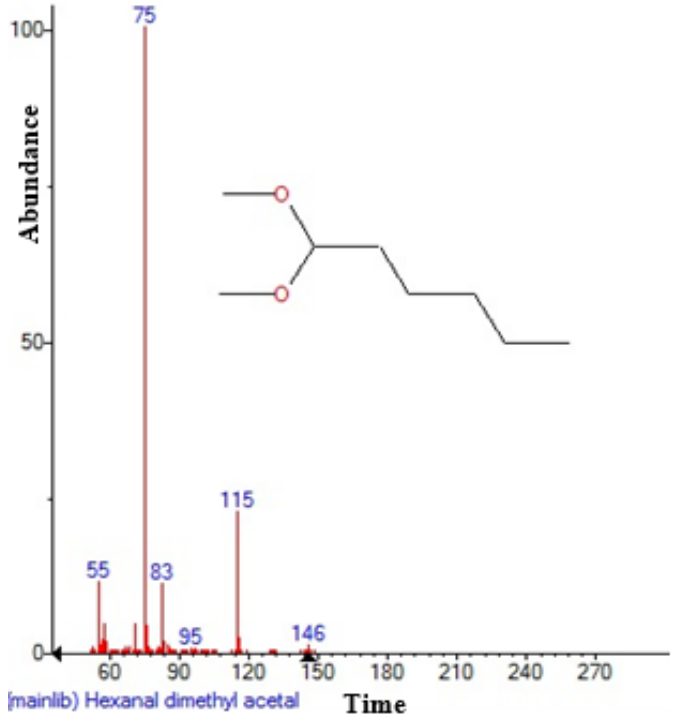


Figure 4. Structure of Hexanal dimethyl acetal with 3.533 (RT) present in *Euphorbia lathyrus*.

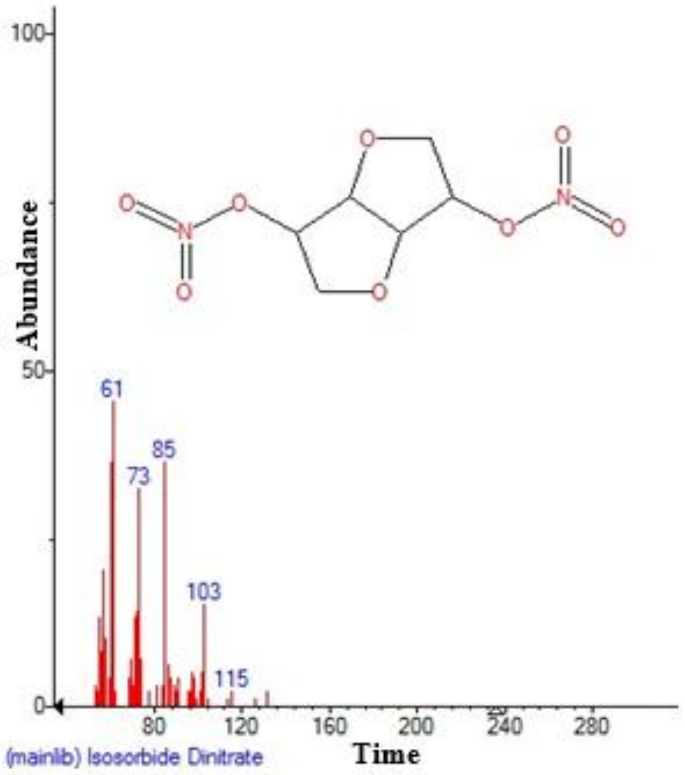


Figure 5. Structure of Isosorbide dinitrate with 4.546 (RT) present in *Euphorbia lathyrus*.

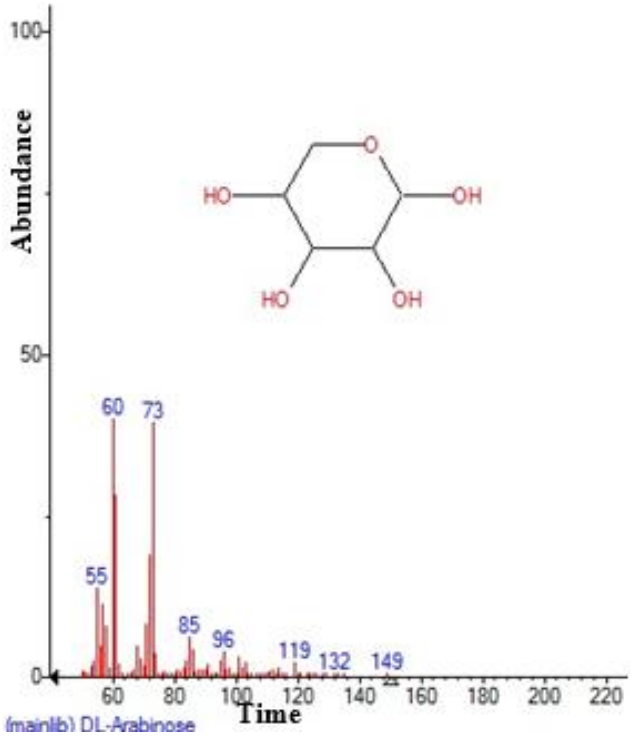


Figure 6. Structure of DL-Arabinose with 4.878 (RT) present in *Euphorbia lathyrus*

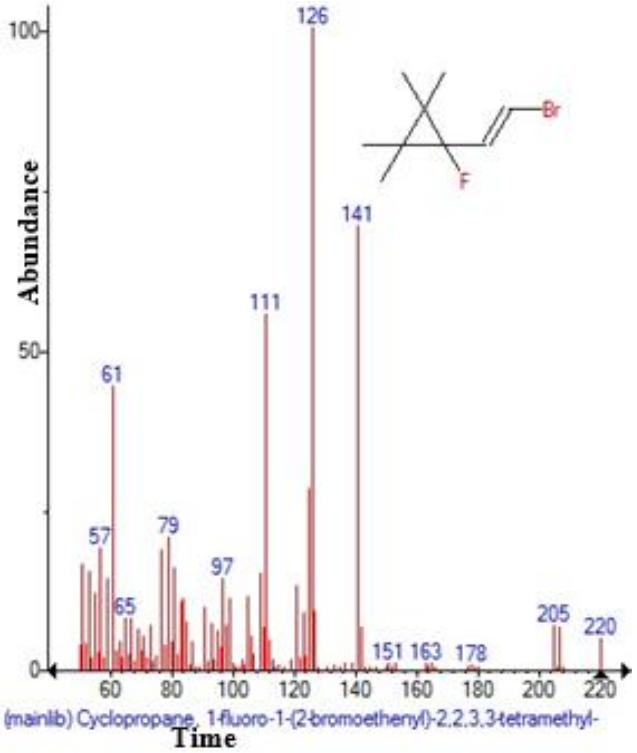


Figure 7. Structure of Cyclopropane ,1-fluoro-1-(2-bromoethenyl)-2,2,3,3-tetramethyl with 5.010 (RT) present in *Euphorbia lathyrus*.

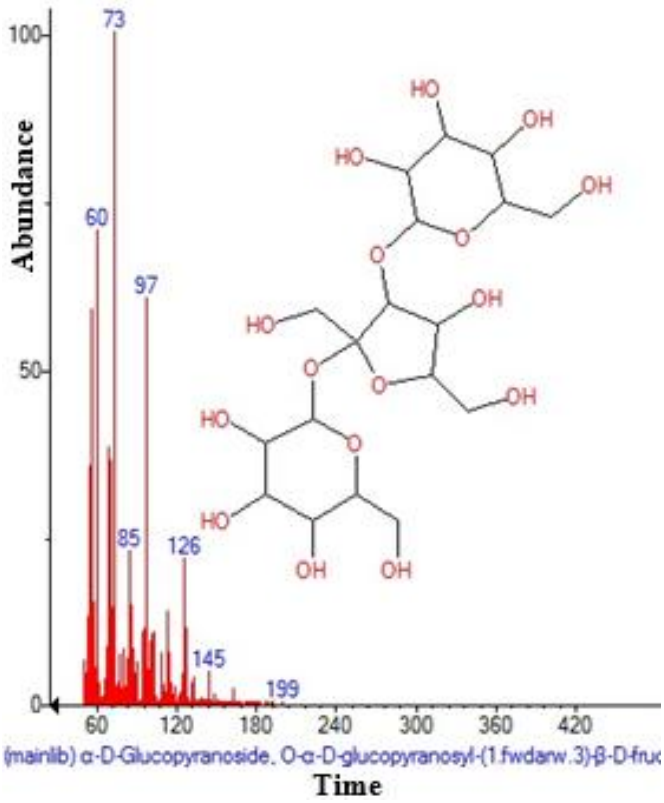


Figure 8. Structure of α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-ß-d-fruc with 5.261 (RT) present in *Euphorbia lathyrus*.

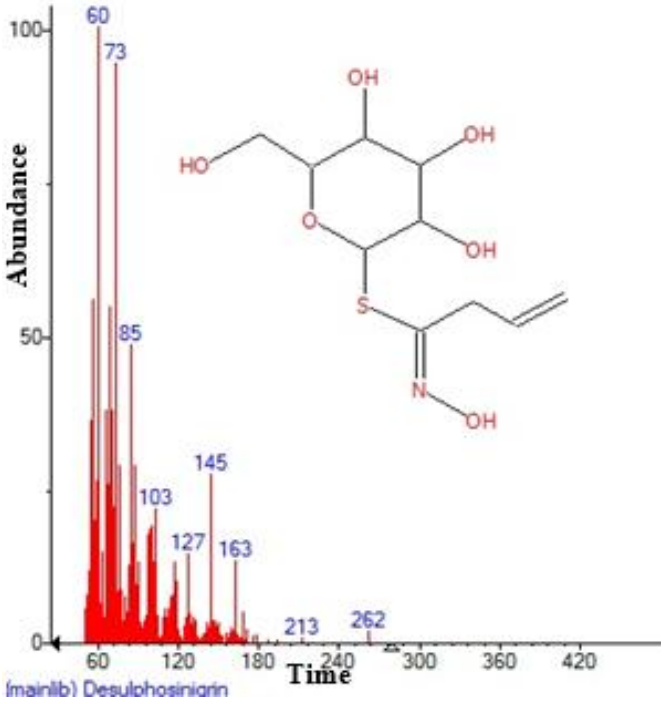


Figure 9. Structure of Desulphosinigrin with 5.313 (RT) present in *Euphorbia lathyrus*.

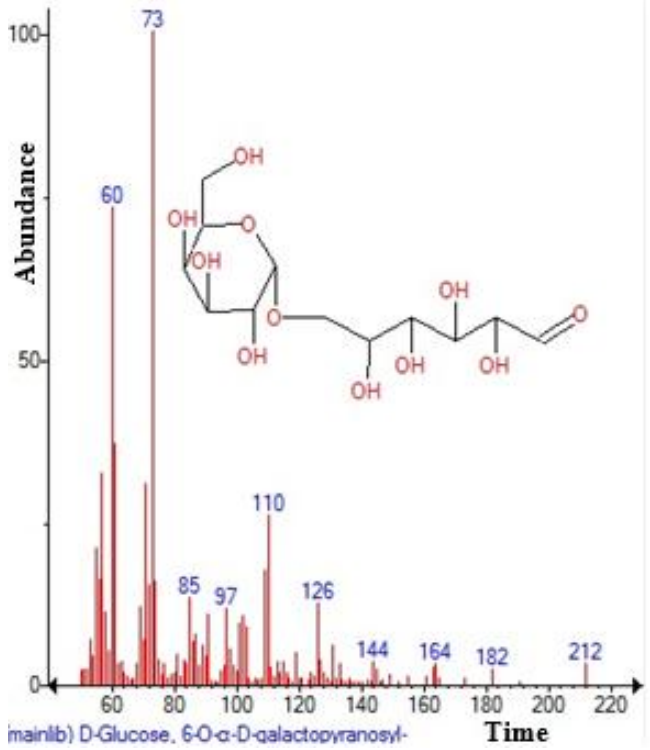


Figure 10. Structure of D-Glucose , 6-O-α-D-galactopyranosyl with 5.782 (RT) present in *Euphorbia lathyrus*.

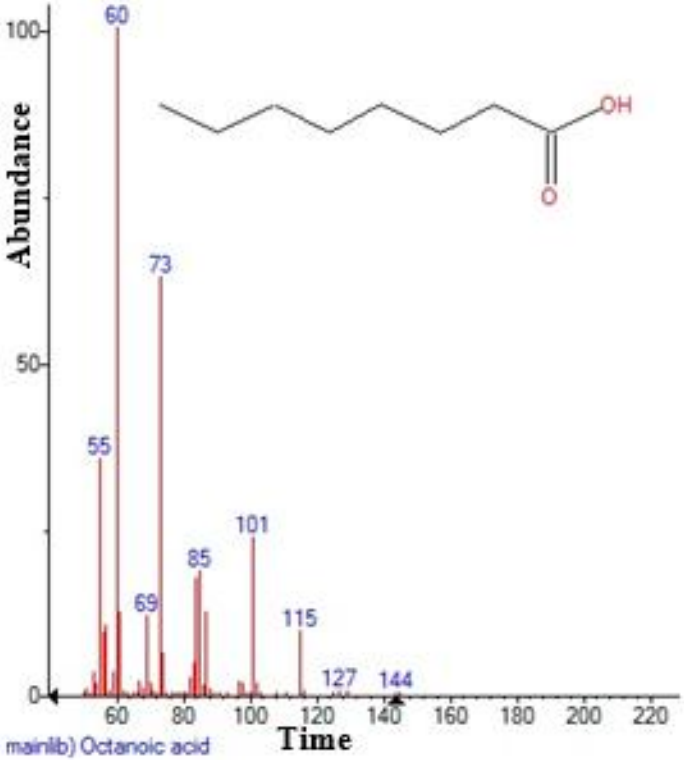


Figure 11. Structure of Octanoic acid with 6.137 (RT) present in *Euphorbia lathyrus*.

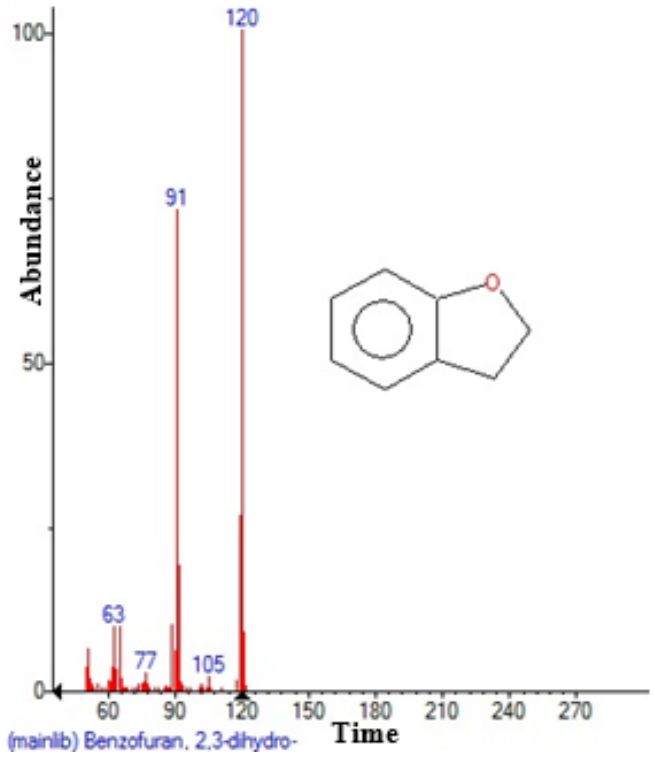


Figure 12. Structure of Benzofuran ,2,3-dihydro with 6.715 (RT) present in *Euphorbia lathyrus*.

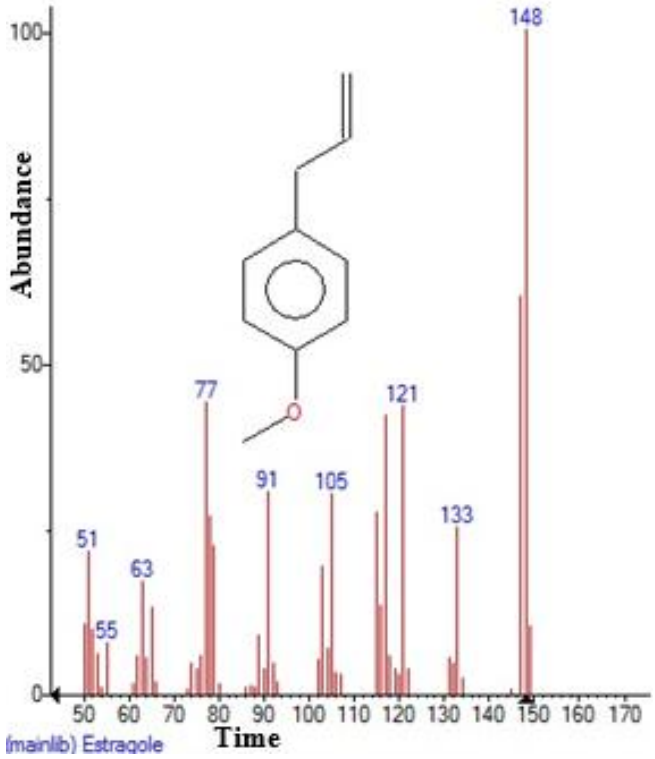


Figure 14. Structure of Estragole with 7.481 (RT) present in *Euphorbia lathyrus*.

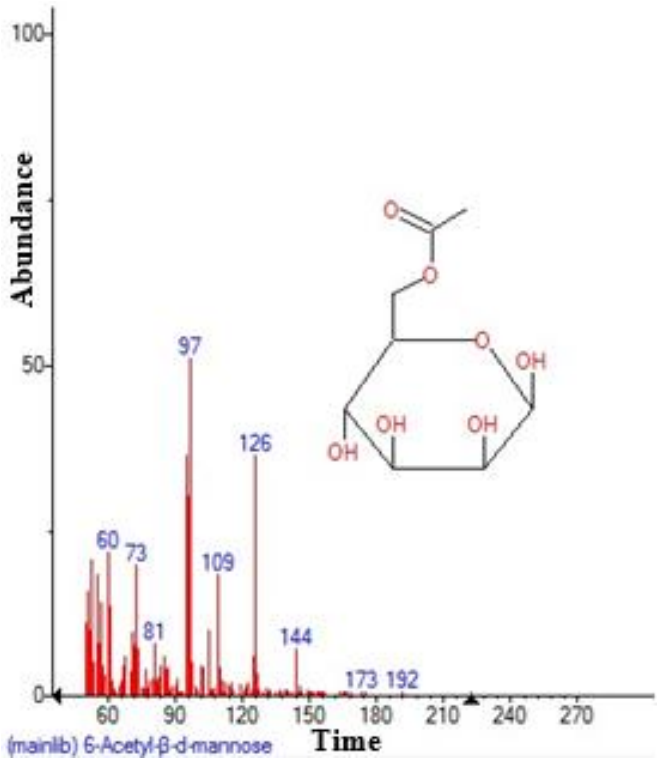


Figure 13. Structure of 6-Acetyl-ß-d-mannose with 6.984 (RT) present in *Euphorbia lathyrus*.

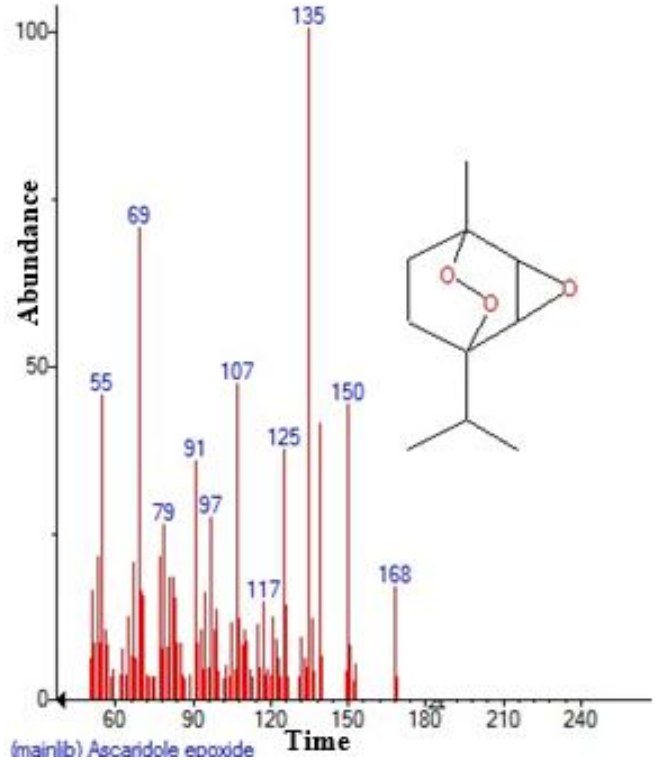


Figure 15. Structure of Ascaridole epoxide with 7.727 (RT) present in *Euphorbia lathyrus*.

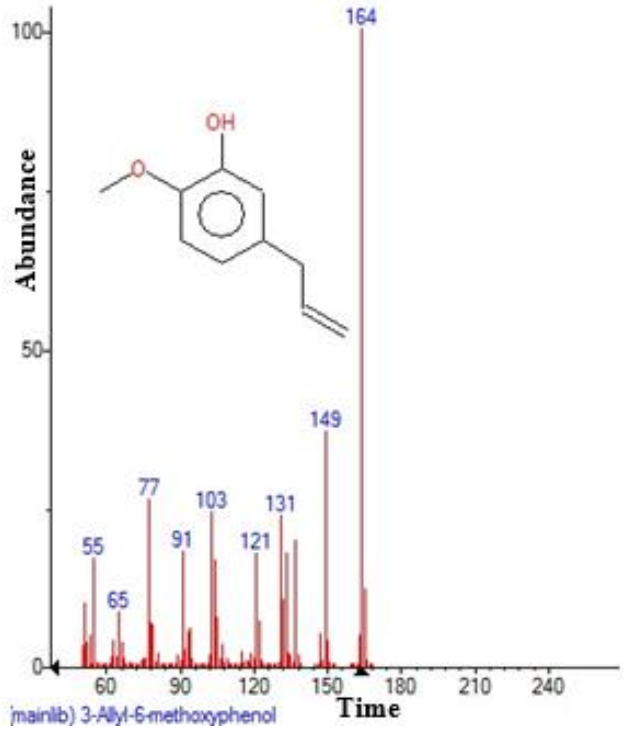


Figure 16. Structure of 3-Allyl-6-methoxyphenol with 8.443 (RT) present in *Euphorbia lathyrus*.

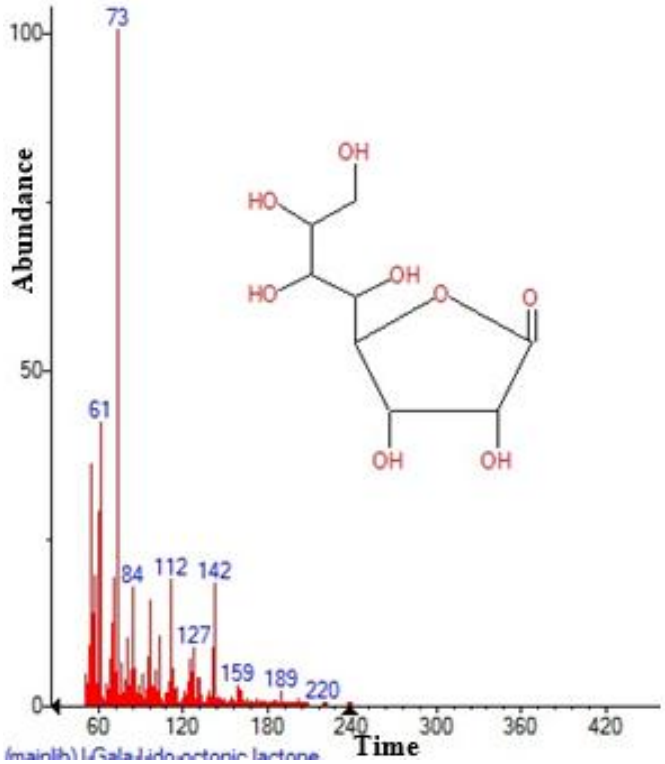


Figure 18. Structure of l-Gala-l-ido-octonic lactone with 10.743 (RT) present in *Euphorbia lathyrus*.

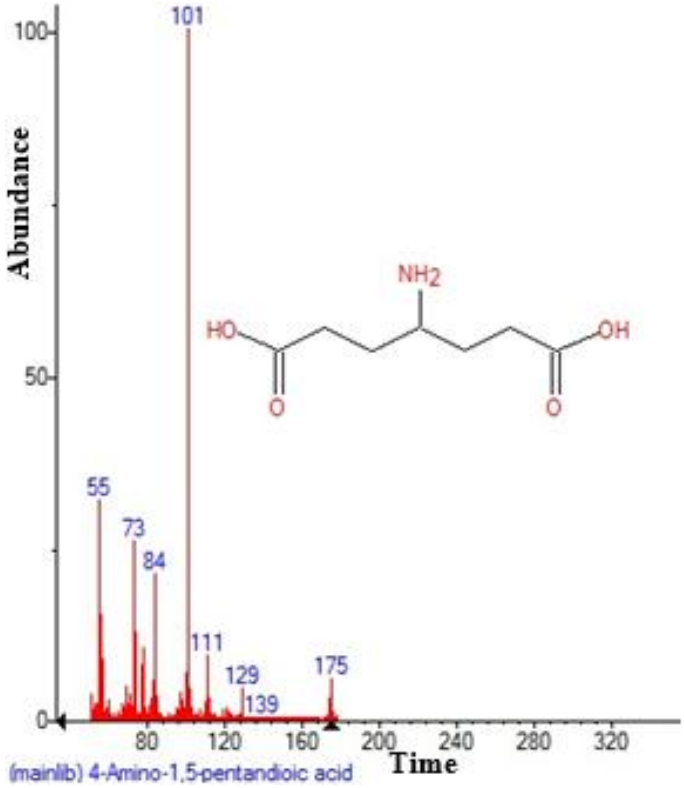


Figure 17. Structure of 4-Amino-1,5,pentandioic acid with 9.564 (RT) present in *Euphorbia lathyrus*.

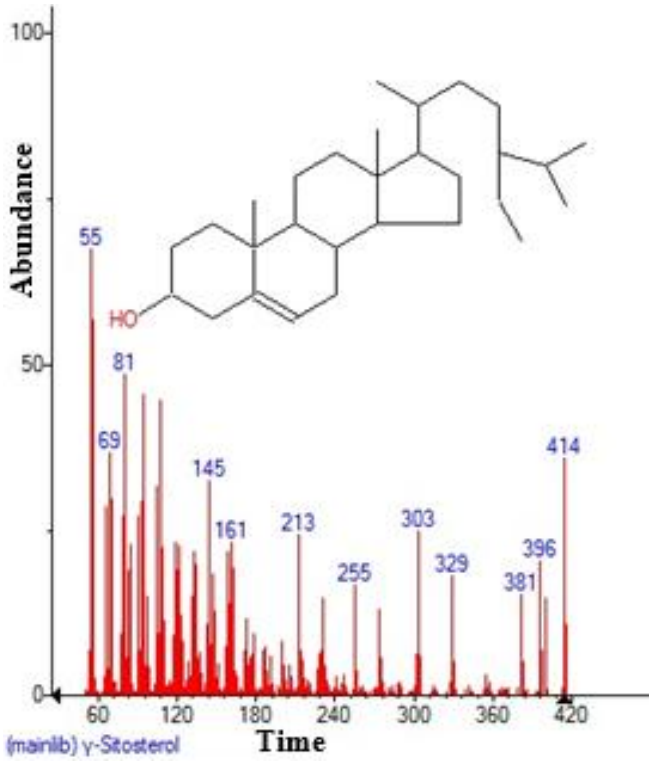


Figure 19. Structure of γ-Sitosterol with 15.023 (RT) present in *Euphorbia lathyrus*.

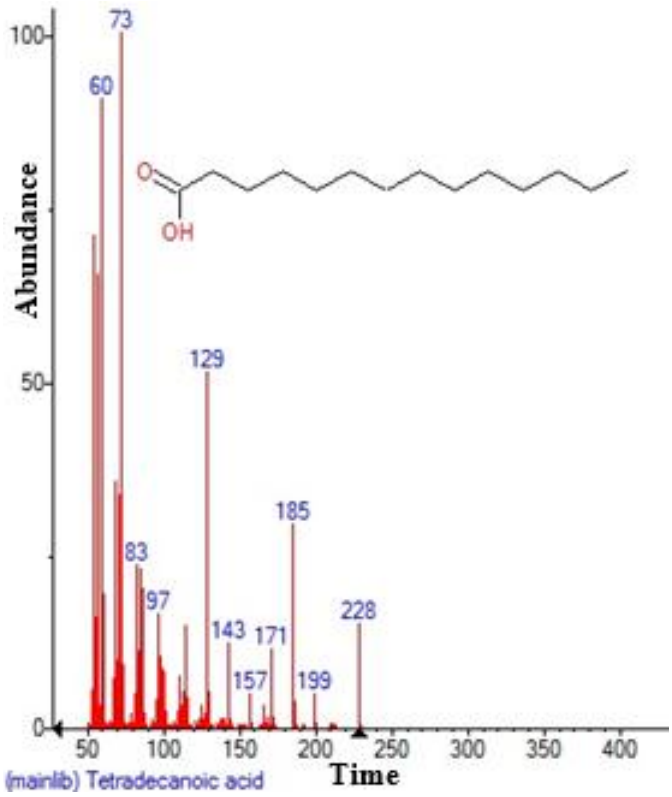


Figure 20. Structure of Tetradecanoic acid with 13.455 (RT) present in *Euphorbia lathyrus*.

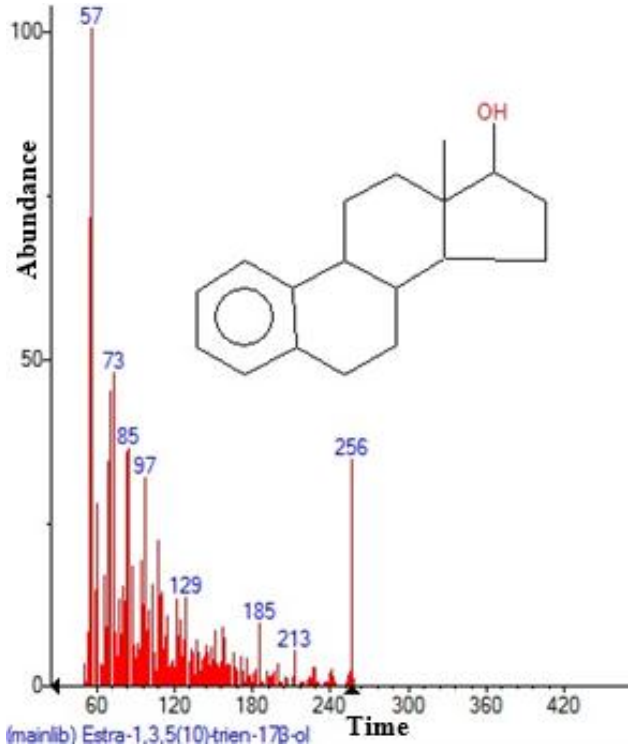


Figure 22. Structure of Estra -1,3,5(10)-trien-17ß-ol with 16.007 (RT) present in *Euphorbia lathyrus*.

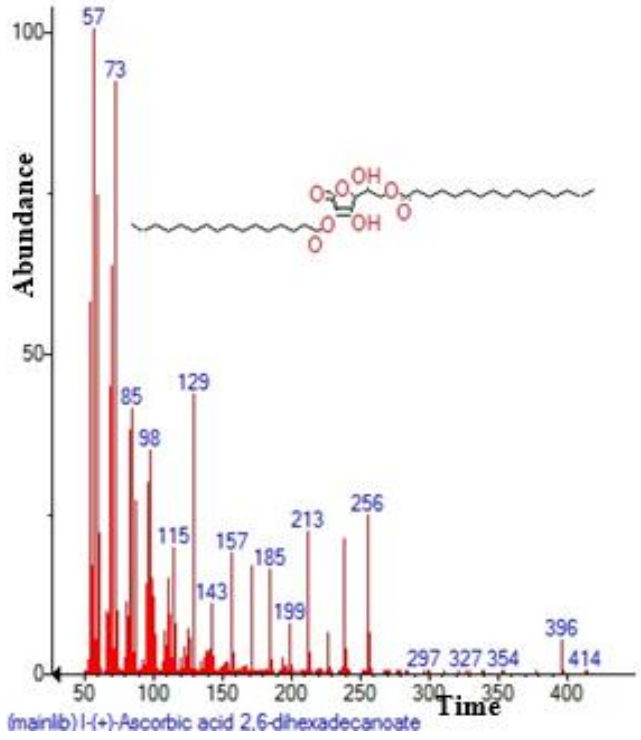


Figure 21. Structure of l-(+)-Ascorbic acid 2,6-dihexadecanoate with 15.486 (RT) present in *Euphorbia lathyrus*.

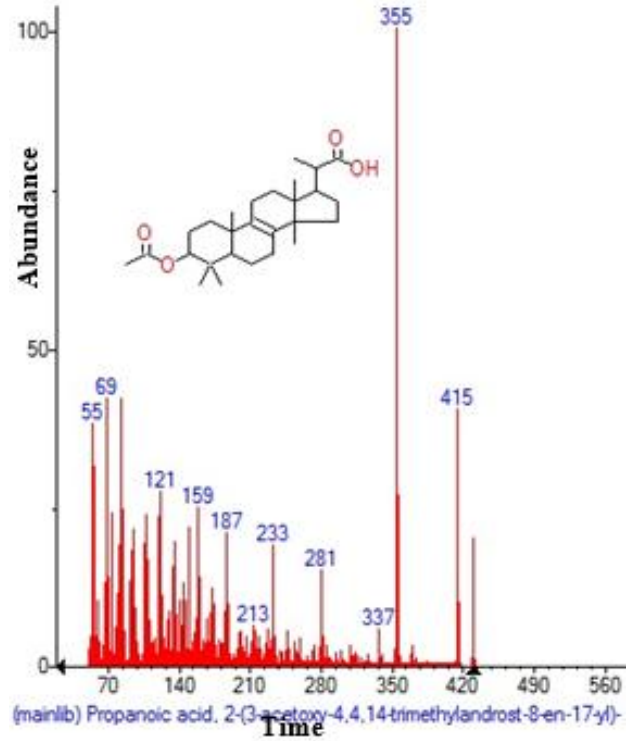


Figure 23. Structure of Propanoic acid ,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) with 16.745 (RT) present in *Euphorbia lathyrus*.

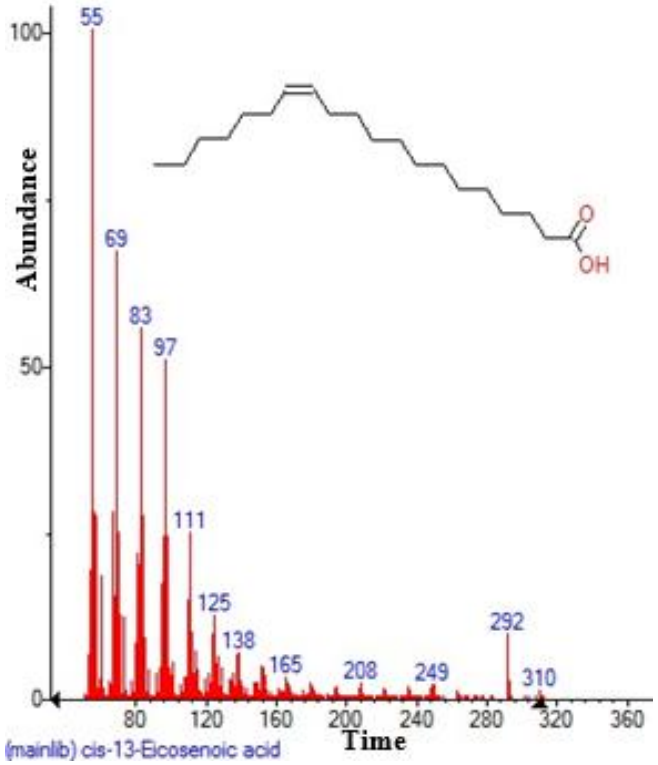


Figure 24. Structure of Cis-13-Eicosenoic acid with 18.914 (RT) present in *Euphorbia lathyrus*.

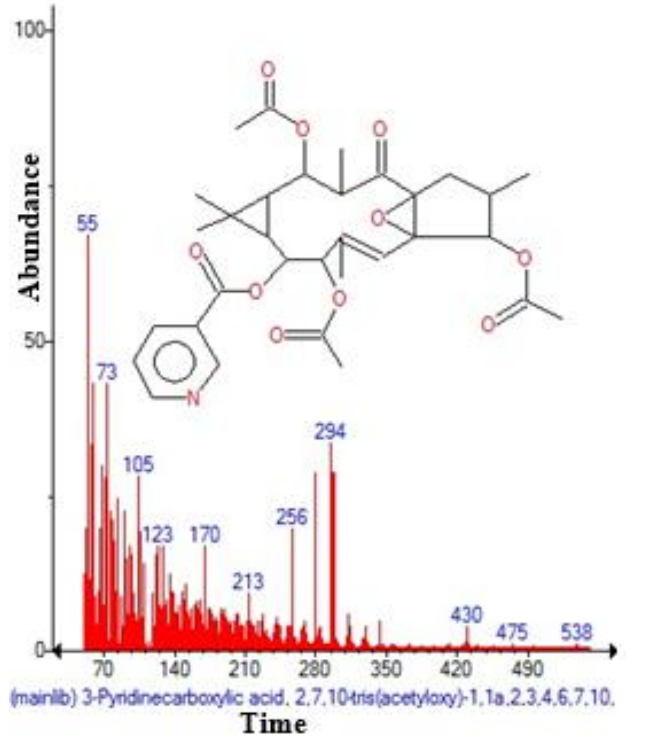


Figure 26. Structure of 3-Pyrinecarboxylic acid , 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10 with 19.246 (RT) present in *Euphorbia lathyrus*.

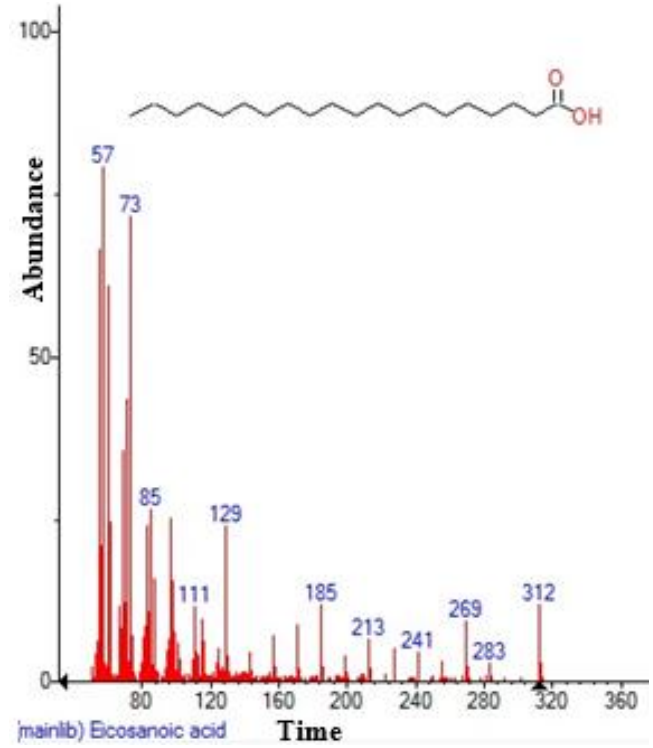


Figure 25. Structure of Eicosanoic acid with 19.051 (RT) present in *Euphorbia lathyrus*.

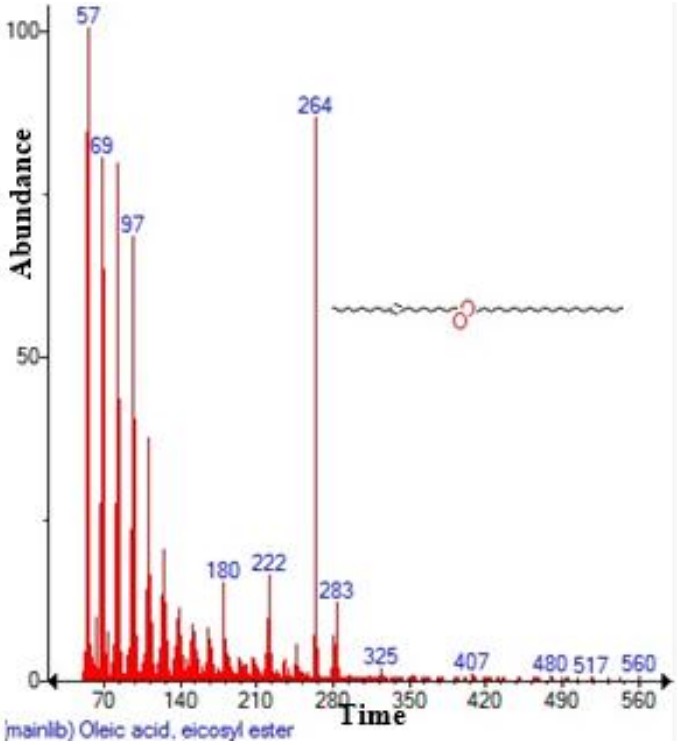


Figure 27. Structure of Oleic acid , eicosyl ester with 20.339 (RT) present in *Euphorbia lathyrus*.

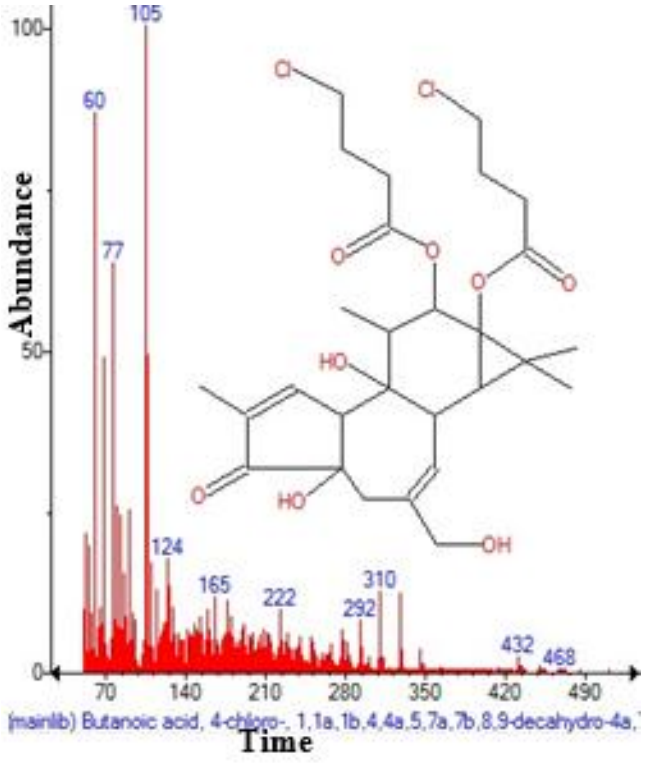


Figure 28. Structure of Butanoic acid , 4-chloro-,1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a with 21.083 (RT) present in *Euphorbia lathyrus*.

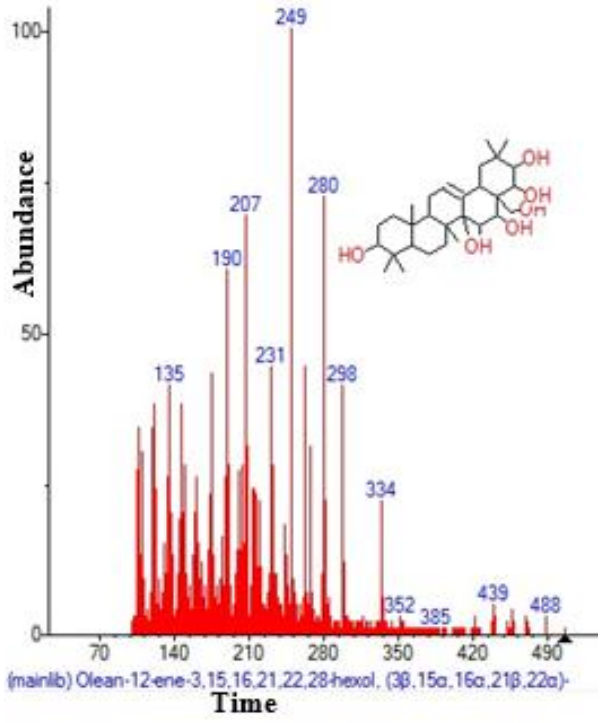


Figure 30. Structure of Olean -12-ene-3,15,16,21,22,28-hexol, (3ß,15α,16α,21ß,22α) with 21.603 (RT) present in *Euphorbia lathyrus*.

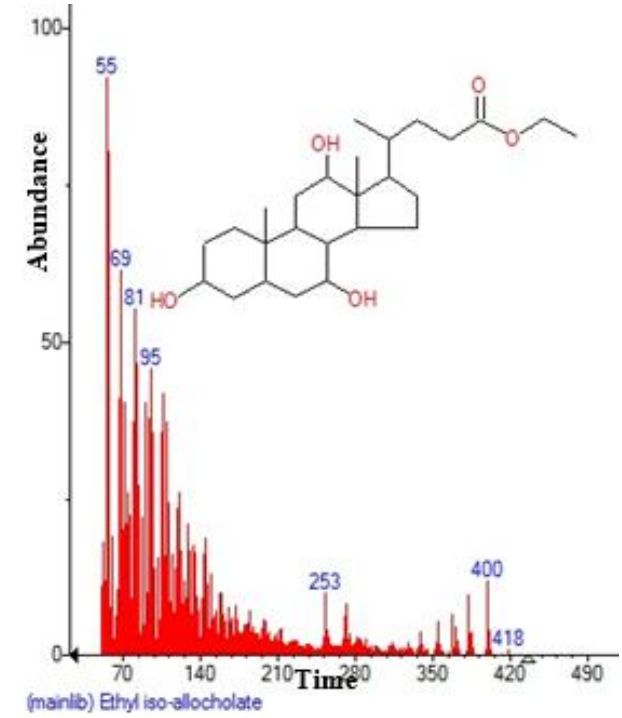


Figure 29. Structure of Ethyl iso –allocholate with 21.134 (RT) present in *Euphorbia lathyrus*.

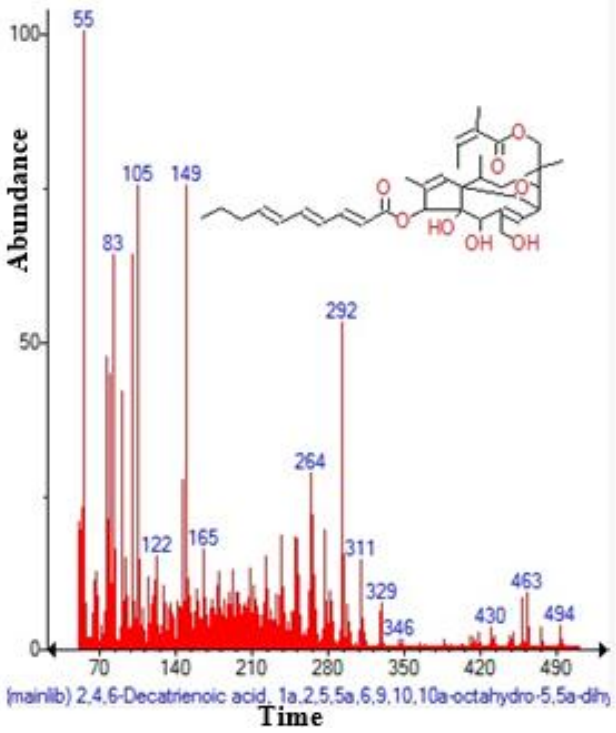


Figure 31. Structure of 2,4,6-Decatrienoic acid ,1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihy with 21.878 (RT) present in *Euphorbia lathyrus*.

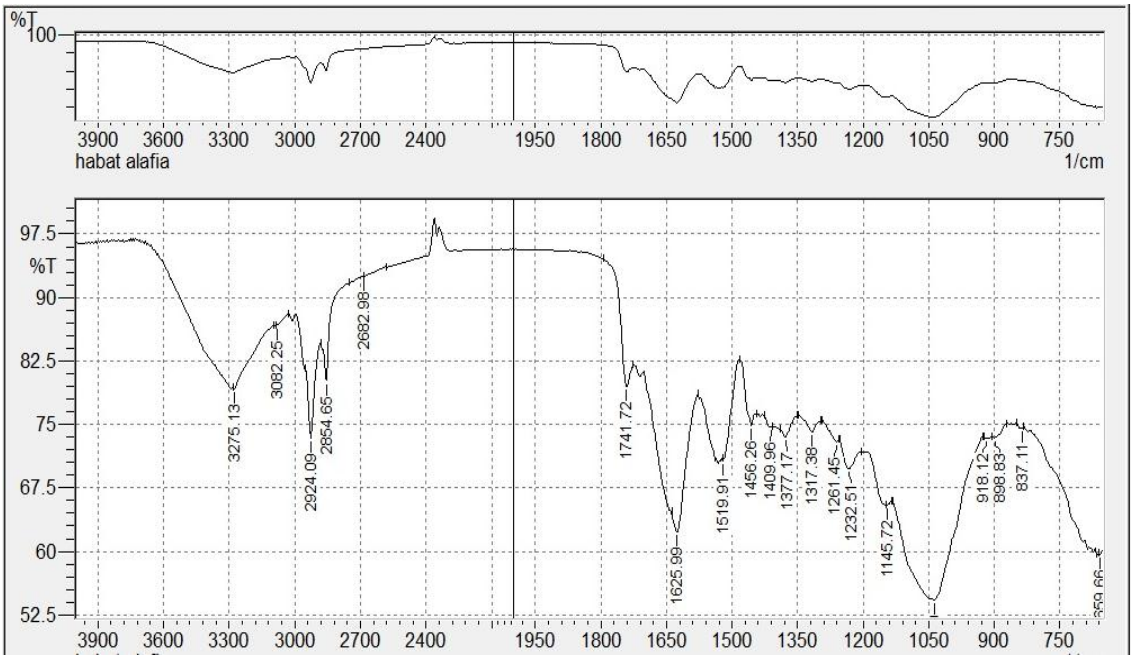


Figure 32. FT-IR profile of *Euphorbia lathyrus.*

Conclusion

E. lathyrus is native plant of Iraq. It contains chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic, and antiasthamatic.

Conflict of interest

The authors have not declared any conflict of interest

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